Leukocyte-rich Platelet rich plasma (L-PRP). Platelets and leukocytes counts were performed on samples of blood, PRGF and L-PRP with a hematology analyzer. Morphological analysis of PRGF and L-PRP scaffolds was performed with scanning electron microscopy (SEM). Mechanical properties of the scaffolds were determined by a tensile test. PRGF and L-PRP scaffolds were incubated in the absence (non-inflammatory conditions) or presence of inflammatory condition. The conditioned media released by the different scaffolds was collected and the concentration of several growths factors and pro-inflammatory cytokines was determined by enzyme-linked immunosorbent assay (ELISA).

Results: Inclusion of the white blood cells in the PRP increased the presence of leukocytes from 0.2 to 19.3. The biological outcomes of the L-PRP under inflammatory conditions were dramatically altered compared with the outcomes showed by the PRGF. In fact, the structure of fibrin network was significantly modified due to the leukocytes and the presence of MMP-1 within the scaffolds increased from 2 to 79 ng/mL. As a consequence the leukocyte containing fibrin membranes were more fragile, reducing both their elongation capacity and the time until membrane rupture in more than 28% compared to PRGF scaffolds. The release of pro-inflammatory cytokines from scaffolds was significantly increased when leukocytes were included in the PRP and an inflammatory condition was evaluated. The amount of TNF-alpha, IL-6, IL-1 and IL-8 released from the L-PRP fibrin scaffolds was 31, 248, 151 and 381-fold higher than from PRGF scaffolds. The inflammatory response of fibroblast was significantly higher to L-PRP condicioned cultures than to PRGF ones.

Conclusions: Compared with PRGF, L-PRP induces significantly higher pro-inflammatory conditions. The inclusion of leukocytes alters fibrin network, reduces its biomechanical properties and increases the presence of pro-inflammatory cytokines such as TNF-alpha, IL-6, IL-1 and IL-8, which deeply influences the cellular inflammatory response.

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MOLECULAR RESPONSES OF THE INFRAPATELLAR FAT PAD MAY CONTRIBUTE TO PATHOLOGICAL CHANGES IN THE KNEE JOINT FOLLOWING IDEALIZED ANTERIOR CRUCIATE LIGAMENT SURGERY.

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Purpose: Severe injuries to the anterior cruciate ligament (ACL) require surgical reconstruction to restore mechanical stability in the knee joint. In spite of successful surgical reconstruction, in some cases the cartilage of the knee joint exhibits signs of degeneration analogous to symptoms related to osteoarthritis (OA). The causes leading to OA even after successful reconstruction remain to be defined. We developed a surgical model of an "idealized reconstruction of ACL" and hypothesized that immediate anatomical reconstruction of ACL will not lead to degeneration of the knee joint components. However, previous examination of synovium, cartilage and the ACL exhibited elevated molecular responses for inflammatory and degradative biomarkers early after the surgery, which subsided by 20 weeks. As the different components of the knee joint function as an integrated coordinated system, in the present study we examined the molecular responses in the infrapatellar fat pad (IPFP). The IPFP is present in the anterior compartment of the knee joint in close proximity to the synovial layers and cartilage surfaces. This suggests that the IPFP may be able to influence the catabolic and inflammatory processes in different components of the knee joint. Previous reports have observed pathological changes in this tissue either following arthroscopic procedures or secondary to knee joint insults such as patellar dislocation and tendonitis. This study was based on the hypothesis that following ACL-R, molecular changes will be initiated in the IPFP that will be sustained over time, thus acting as a molecular repository for fibrotic processes in the joint.

Methods: In this study, for analysis of IPFP tissue samples at 2 and 20 weeks, a total of 21 skeletally mature (3–4 year old) female Suffolk-cross sheep were allocated to 3 groups: a) 2 week ACL-R surgical group (N = 9), b) 20 week ACL-R surgical group (N = 7) and c) non-operated control group (N = 5). These tissues were assessed by real time q-PCR for mRNA levels of select molecules involved in remodelling and synthesis following surgery such as Collagen type I (Col-I), collagen Type III (Col-III) and associated growth factors such as transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF). The mRNA expression levels were normalized to 18S mRNA. ANOVA with Bonferroni post-hoc analysis was used to determined differences in expression levels between groups, using SPSS 19.0.

Results: Histological analysis of IPFP revealed a fibrogenic reaction of this tissue following ACL-R that was evident at 2 weeks post-injury. Molecular analysis revealed that the mRNA levels for TGF-β were elevated at 2 weeks following ACL-R surgery, but returned to levels close to the un-operated controls by 20 weeks. The state of the tissue correlates well with this molecular marker as it has been reported that TGF-β is a potent inducer of processes observed in fibrosis. VEGF is another growth factor that plays a major role in angiogenesis and was found to be elevated in the IPFP tissue in the early time points after ACL-R. Interestingly, elevations in Type I and Type III collagen were also observed in this fat pad, again consistent with a fibrogenic process in this tissue. The pattern of expression at the 2 and 20-week time points were similar to that of the growth factors discussed above.

Conclusion: The present study suggests that anabolic factors are activated and expressed in the IPFP tissue in a similar pattern as observed previously for synovium and cartilage in the same model. The initial fibrotic response did not resolve by 20 weeks post-injury, possibly compromising the function of the IPFP in the long term.

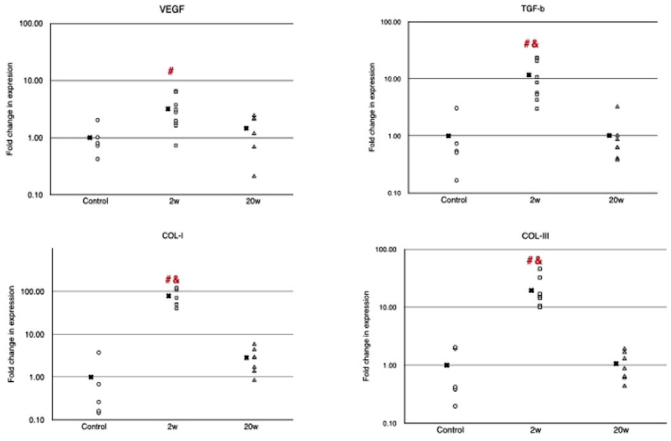

Fig. 1. Fold change expression levels of inflammatory mediators and adipokines measured infrapatellar fat pad after idealized ACL reconstruction in a sheep knee. Levels are normalized to 18S rRNA. Levels are normalized to 18S rRNA.

X – represents the moan expression level for each group and marker shown
○ □ Δ - represent a single measure from each sample in the group
- Significant difference between Control and 2w expression levels
& - Significant difference between 2 w and 20 weeks
@ - Significant difference between Control and 20 weeks

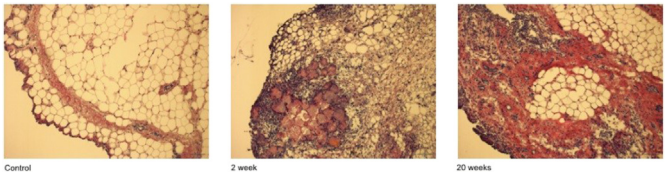

Fig. 2. – H&E images of infrapatellar fat pad after ACL reconstruction 10× magnification

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DIFFERENT DISTRIBUTION AND ACTIVATION DEGREE OF TH17 CELLS IN PERIPHERAL BLOOD IN PATIENTS WITH OSTEOARTHRITIS, RHEUMATOID ARTHRITIS AND HEALTHY DONORS: PRELIMINARY RESULTS OF THE MAGENTA CLICAO (CLINICAL CELL ANALYSIS IN OSTEOARTHRITIS) STUDY

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Background: Osteoarthritis (OA) as well as rheumatoid arthritis (RA) are chronic diseases associated with joint destruction and mobility impairment. Data about changes in immune system in RA and the significant